

MEMORANDUM OF CONFERENCE

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**Subject:** High Oleic Acid Transgenic Soybean

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**Introduction**

This meeting was intended to bring to closure DuPont's consultation with FDA, which started on January 24, 1995. DuPont submitted a summary to support their safety and nutritional assessment of their new transgenic soybean varieties on August 28, 1996. Additional information to address potential safety concerns regarding the presence of an ampicillin resistance marker ( $\beta$ -lactamase) gene in their transgenic soybean was submitted by DuPont on October 16, 1996.

**Intended Effect and Food/Feed Use**

The intended technical effect of this genetic modification of soybean, *Glycine max*, plants is to produce soybeans with a greatly increased oleic acid content in their oils.

According to DuPont, their soybean varieties, called high oleic acid transgenic soybeans, have been modified to suppress the endogenous expression of the GmFad2-1 gene which encodes a  $\delta$ -12 desaturase enzyme. These soybean lines are homozygous for a GmFad2-1 cDNA in the sense

orientation under the control of a seed-specific promoter. The inserted GmFad2-1 cDNA causes a coordinated silencing of itself and the endogenous GmFad2-1 gene. This suppression of endogenous GmFad2-1 gene product expression prevents the addition of a second double bond to oleic acid in the endogenous fatty acid synthetic pathway in soybean seeds, leading to increased levels of oleic acid in the seeds.

Soybean plants are grown for their seed, which is further processed to yield oil and meal. Soybean oil is currently the predominant plant oil in the world, and is used in a wide variety of food applications. Untreated, soybean oil is rich in polyunsaturated fatty acids, which are oxidatively unstable, making it unsuitable for many food applications. To enhance stability, selective hydrogenation, a chemical process which greatly decreases the content of polyunsaturated fatty acids, mainly linoleic and linolenic acids, and increases the relative abundance of monounsaturated oleic acid, has been employed by the food industry; however, this process results in the production of substantial quantities of the trans isomers of oleic acid and other fatty acids. In contrast, high oleic acid soybean oil obtained from transgenic soybeans, does not contain trans isomers.

According to DuPont, conventional breeding techniques (mutational breeding) have been used to produce soybeans with elevated oleic acid content (35%-55%); however, the high oleic phenotype is variable depending on environmental conditions. The transgenic soybean varieties are reported to have stable phenotypes.

### **Molecular Alterations and Characterization**

DuPont reported that they introduced two constructs into the meristems of an elite soybean line by particle bombardment producing soybean line 260-05. One of the constructs contained the GmFad2-1 sense cDNA, the other a dihydrodipicolinic acid synthase (dapA) gene with a promoter for enhanced expression in seeds. Expression of the dapA gene under these conditions should lead to an increase in the free lysine content of the seed. According to the information provided by DuPont, the GmFad2-1 incorporated into the soybean genome at two different loci, either at both loci or only one loci, in the R1 seeds from the original R0 260-05 transformant (lines G168 and G94), while the dapA gene only incorporated along with the GmFad2-1 at one loci (line G94). In the case of the R1 plants that had genes incorporated into their genome at both loci (line G94), DuPont selected R2 seeds in which the dapA gene and its controlling sequences were lost by segregation. DuPont presented data from two lines, G94-1 and G94-19, derived from the R2 seeds in which the dapA gene and its controlling sequences were lost by segregation. The sublines that are the subject of this consultation are derived from the original soybean line 260-05, are homozygous for the locus containing the gene silencing GmFad2-1, and devoid of a second locus which contained two over-expressing genes, the GmFad2-1 and dapA genes (lines G168, G94-1 and G94-19). DuPont provided information indicating that these sublines produce a

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soybean oil with a content of oleic acid exceeding 80% and considers these sublines to be isogenic, referring to them as high oleic acid transgenic soybeans.

According to DuPont, GmFad2-1, which is normally expressed only in the developing seed, is under the control of a strong, seed-specific promoter derived from the  $\alpha'$ -subunit of the soybean  $\beta$ -conglycinin gene. This promoter allows high level seed specific expression of the trait gene. A 3' fragment (1174 base pairs) from the phaseolin gene of green bean was used to terminate the transcription. For maintenance and replication in *E. coli*, the vector with the GmFad2-1 gene also contained *E. coli* regulatory sequences and the complete  $\beta$ -lactamase gene for bacterial selection with the antibiotic ampicillin. The  $\beta$ -lactamase gene is reported not to be expressed in the transformed plants. To identify the transformed soybean plants the selectable marker gene,  $\beta$ -glucuronidase (GUS) from *E. coli* under control of the Cauliflower Mosaic Virus 35S promoter, was used.

DuPont presented a summary of Southern blot analyses and Northern blot analyses to demonstrate that the high oleic acid transgenic soybean contained two additional copies of the GmFad2-1 gene and that this genetic change led to the suppression of transcription of the GmFad2-1 gene. The expression levels of GmFad2-2 gene, a second endogenous "housekeeping"  $\delta$ -12 desaturase gene, which is expressed throughout the plant at a constant level, is reported to be only slightly decreased in the transgenic plants. Likewise, DuPont presented data indicating that although the  $\beta$ -glucuronidase (GUS) gene was present in the transgenic plant's genome, GUS expression was absent from the leaves and seeds, as illustrated by Northern and Western blot analyses. Because the  $\beta$ -lactamase gene is under control of a bacterial promoter, it is also not expressed in the transgenic plants as indicated by lack of mRNA transcript and  $\beta$ -lactamase enzymatic activity. Thus, DuPont concludes that the expression of the GmFad2-1 gene is suppressed at the transcriptional level and no new proteins are expressed in this transgenic plant. Based on the Southern blot profile, DuPont indicates that the integrated genetic insert is present at the same position in the transgenic soybean genome over at least four generations. In addition, DuPont observed that the genetic trait had been stable for six generations in the field trials.

In DuPont's submission of October 16, 1996, DuPont addressed the potential for horizontal transfer of the  $\beta$ -lactamase (ampicillin resistance) marker gene to gut and rumen microorganisms. DuPont noted that this issue for the case of the kanamycin resistance marker gene was addressed by FDA in a final rule (59 FR 26700) for the use of aminoglycoside 3'-phosphotransferase II (APH(3')II) as a processing aid in the development of new varieties of tomato, oilseed rape and cotton. For the case with the ampicillin resistance gene, DuPont stated that (1) an intact origin of replication and ampicillin resistance sequence is extremely unlikely to survive in an intact form the hostile environment of an animal gut or rumen; (2) even if such an event were to occur, the possibility of the DNA reaching a competent cell and undergoing natural transformation is extremely unlikely; and (3) even if the first two improbable events were to occur, it would be

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virtually impossible for the DNA to autonomously replicate or be maintained and expressed in a ruminal or gut organism. Based on these reasons, DuPont concluded that the chance of transfer of the ampicillin resistance gene to microorganisms in the animal gut was infinitesimally small.

#### **Compositional Analysis**

The intent of the genetic modification made by DuPont was to modify the fatty acid composition of soybean oil. DuPont did not anticipate any other effect. However, to preclude the possibility that an unintended effect may have rendered either the meal or oil unsafe or inferior, DuPont conducted analytical tests on the composition of soybeans from the transgenic soybean lines and compared the results to the parental line used in construction of the transgenic plants. Two different growth conditions were used for each of the soybean lines and soybeans from both growth conditions were tested.

#### Anti-nutrients

DuPont presented data from analyses with high oleic and control soybeans for the anti-nutritional factors, trypsin inhibitor, phytic acid, raffinose and stachyose. DuPont concluded that no differences were observed between the parental control and the transgenic soybeans for the anti-nutritional factors. Also, DuPont reported the range in concentration of these anti-nutritional factors for a wide variety of commodity soybean lines. The concentration of the anti-nutritional factors in the transgenic soybeans were within the range for the commodity soybean lines.

#### Allergenicity

DuPont observed changes in levels of  $\beta$ -conglycinin and glycinin seed storage proteins in high oleic acid transgenic soybeans when compared to parental soybeans. A similar reduction of  $\alpha$ -type subunits of  $\beta$ -conglycinin has been observed in some naturally occurring soybean varieties and in soybeans with conventionally-induced mutations. DuPont presented data indicating that this effect was a result of promoter cosuppression resulting in a low level of  $\beta$ -conglycinin  $\alpha$  and  $\alpha'$  subunits and a high level of the acidic (A) and basic (B) subunits of glycinin and the precursor to the A2 and B1A glycinin subunits in the modified seeds to counter the decrease in levels of  $\beta$ -conglycinin subunits. Although DuPont observed changes in the seed storage proteins, the firm states that total protein and amino acid levels in transgenic soybean meal were unaffected by these changes.

Because of these differences in the protein profile between the high oleic acid transgenic soybeans and the parental soybeans, DuPont considered the allergenic potential of the high oleic acid soybeans. Using sera from a number of human subjects who demonstrated the classic symptoms of an allergy to soybeans, DuPont performed radioallergosorbent tests (RAST), RAST inhibition experiments and immunoblot analysis with the high oleic acid transgenic soybeans and the parental

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soybeans. From these tests, no difference was observed in soybean specific IgE reactivity between the high oleic acid transgenic soybeans and the parental soybeans. In addition, DuPont reported data from a study presented at the Institute of Food Technology (IFT) 1996 meeting in which 67 soybean lines representing 95% of the genetic base in the U. S were examined for soybean specific IgE reactivity. All 67 soybean lines exhibited IgE reactivity. DuPont concludes from their investigation of possible changes in allergenicity between their transgenic soybean varieties and the parental line that there is no significant quantitative or qualitative difference between the transgenic and elite soybeans with regard to their allergen content.

### Nutrients and Isoflavones

DuPont presented data on the nutrient profile of soybeans from the transgenic plants compared to the parental plant. Comparison of the bulk components protein, oil, crude fiber, carbohydrate, and ash content were made. The amino acid composition of transgenic and parental soybeans was also compared. DuPont reported that the nutrient composition of the transgenic soybeans does not differ significantly from the parental soybeans and the values are within the range of those reported for commodity soybeans.

Soybeans contain several biologically active phytoestrogenic isoflavones. DuPont presented data on analyses of the major soybean isoflavones, daidzein, genistein and glycitein from high oleic and control soybeans and reported the range in concentration of these major isoflavones for a variety of commodity soybeans. DuPont concluded that no differences were observed between the parental and transgenic soybeans in the concentration of isoflavones.

### Fatty Acid Composition

The intended effect of DuPont's modification is to alter the fatty acid composition of soybean oil. DuPont reports that the oil resulting from the genetic modification (High Oleic Soybean Oil) does contain a fatty acid profile that is very different from those found in commodity soybean oil. Oleic acid makes up over 80% of total fatty acids, substantially higher than that found in traditional soybean oil. Linoleic acid content is approximately 1% which is substantially lower than standard for traditional soybean oil. Likewise, linolenic acid and palmitic acid content are lower than standard for traditional soybean oil. Also, DuPont reported the presence of a small amount, less than 1% of total fatty acids, of a 9,15 isomer of linoleic acid normally found only in hydrogenated soybean oils. This isomer is present in hydrogenated soybean oils and other foodstuff, such as cheese, beef, human milk, and mango pulp. All other fatty acids detected in the high oleic soybean oil were present at similar relative abundance to those found in traditional soybean oil.

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Due to the changes in fatty acid composition, High Oleic Soybean Oil does not meet the specifications for soybean oil in the Food Chemical Codex (FCC, Committee on Food Chemical Codex, 1996). Therefore, DuPont proposed to FDA the common or usual name "High Oleic Soybean Oil" for oil derived from the new soybean varieties.

### **Conclusions**

Based on the safety and nutritional assessment DuPont has conducted, DuPont has concluded, in essence, that the new soybean varieties, G94-1, G94-19, and G168, that they have developed are not materially different in any respect relevant to food or animal feed safety from soybean varieties currently on the market and that these new soybean varieties do not raise issues that would require premarket review or approval by FDA. DuPont also concluded that based on the compositional differences of the oil and its intended use, as a replacement for hydrogenated soybean oils, that a new common or usual name (High Oleic Soybean Oil) is appropriate to distinguish this oil from soybean oil as defined by FCC. At this time, based on DuPont's description of its data and analyses, the Agency considers DuPont's consultation on these varieties to be complete.

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